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□1: *Plant J* 1995 May;7(5):771-84

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Co-expression of native and introduced genes reveals cryptic regulation of HMG CoA reductase expression in Arabidopsis.

Re EB, Jones D, Learned RM

Section of Molecular and Cellular Biology, University of California, Davis 95616, USA.

In eukaryotes, all isoprenoid compounds share a common precursor, mevalonic acid, whose synthesis is catalyzed by the enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase. As one step towards a better understanding of the role that this enzyme plays in coordinating isoprenoid biosynthesis in plants, Arabidopsis thaliana HMG CoA reductase was ectopically expressed in transgenic Arabidopsis plants. By using this molecular genetic approach, several novel and fundamental observations have been made regarding isoprenoid biosynthesis in Arabidopsis. First, it was demonstrated that the overexpression of authentic Arabidopsis HMG CoA reductase is not sufficient to alter the bulk synthesis and accumulation of the abundant end products of the plant isoprenoid pathway. Second, active transcription of the transgene appears to co-activate and deregulate expression of the native gene. resulting in a striking elevation of HMG CoA reductase mRNA levels. Finally, although very high levels of HMG CoA reductase mRNA were expressed in these transgenic plants, only modest increases in enzyme activity could be detected. Taken together, these data suggest that HMG CoA reductase expression is regulated at multiple levels in plants as well as animals, and they provide a foundation for elucidating the molecular mechanisms for mevalonate regulation in A. thaliana.

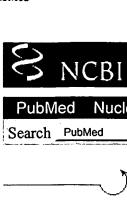
PMID: 7773308, UI: 95291337

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□ 1: Science 1991 May 3;252(5006):675-9

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Modular organization of genes required for complex polyketide biosynthesis.

Donadio S, Staver MJ, McAlpine JB, Swanson SJ, Katz L

Department of Corporate Molecular Biology, Abbott Laboratories, Abbott Park, IL 60064.

In Saccharopolyspora erythraea, the genes that govern synthesis of the polyketide portion of the macrolide antibiotic erythromycin are organized in six repeated units that encode fatty acid synthase (FAS)-like activities. Each repeated unit is designated a module, and two modules are contained in a single open reading frame. A model for the synthesis of this complex polyketide is proposed, where each module encodes a functional synthase unit and each synthase unit participates specifically in one of the six FAS-like elongation steps required for formation of the polyketide. In addition, genetic organization and biochemical order of events appear to be colinear. Evidence for the model is provided by construction of a selected mutant and by isolation of a polyketide of predicted structure.

PMID: 2024119, UI: 91220065

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Filipilite Service		Structure and functional analysis of a marine bacterial carotenoid biosynthesis gene cluster and astaxanthin biosynthetic pathway proposed at the gene level.
		Misawa N, Satomi Y, Kondo K, Yokoyama A, Kajiwara S, Saito T, Oht T, Miki W
	NEW	Central Laboratories for Key Technology, Kirin Brewery Co., Ltd., Kanagav Japan.
Karak Pada		A carotenoid biosynthesis gene cluster for the production of astaxanthin was isolated from the marine bacterium Agrobacterium aurantiacum. This cluster contained five carotenogenic genes with the same orientation, which were designated crtW, crtZ, crtY, crtI, and crtB. The stop codons of individual crt genes except for crtB overlapped the start codons of the following crt genes. Escherichia coli transformants carrying the Erwinia uredovora carotenoid biosynthesis genes provide suitable substrates for carotenoid biosynthesis. The functions of the five crt genes of A. aurantiacum were determined through chromatographic and spectroscopic analyses of the pigments accumulated in some E. coli transformants carrying various combinations of the E. uredovor and A. aurantiacum carotenogenic genes. As a result, the astaxanthin biosynthetic pathway is proposed for the first time at the level of the biosynthesis genes. The crtW and crtZ gene products, which mediated the oxygenation reactions from beta-carotene to astaxanthin, were found to have low substrate specificity. This allowed the production of many presumed intermediates of astaxanthin, i.e., adonixanthin, phoenicoxanthin (adonirubit canthaxanthin, 3'-hydroxyechinenone, and 3-hydroxyechinenone.
		PMID: 7592436, UI: 96062243

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	structure, and chromose	omai localization.
	Her C, Aksoy IA, Kimura S RM	, Brandriff BF, Wasmuth JJ, Weinshilboum
	Department of Pharmacology 55905, USA.	, Mayo Medical School, Rochester, Minnesota
NEW	Sulfation is an important path	way in the metabolism of estrogens. We recently
Per nucyes	cloned a human liver estroger determined the structure and of as a step toward molecular ge STE spans approximately 20 from 95 to 181 bp. The location STE are identical to those four in a rat PST gene. In addition conserved in the human dehyological to the site of transcription initiated cDNA ends. STE was mapped.	n sulfotransferase (EST) cDNA. We have now chromosomal localization of the EST gene, STE, enetic studies of the regulation of EST in humans. kb and consists of 8 exons, ranging in length ons of most exon-intron splice junctions within and in a human phenol ST (PST) gene, STM, and the locations of five STE introns are also droepiandrosterone (DHEA) ST gene, STD. The nations one CCAAT and two TATA sequences. TATA box elements is in excellent agreement with the ston as determined by 5'-rapid amplification of d to human chromosome 4q13.1 by fluorescence and structural characterization of STE will now

PMID: 8530066, UI: 96079087

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1: *J Bacteriol* 1993 Apr;175(8):2197-204

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PubMed Services Genetic construction and functional analysis of hybrid polyketide synthases containing heterologous acyl carrier proteins.

Khosla C, McDaniel R, Ebert-Khosla S, Torres R, Sherman DH, Bibb MJ, Hopwood DA

Department of Genetics, John Innes Institute, John Innes Centre, Norwich, United Kingdom.

Notation Resources

NEW

The gene that encodes the acyl carrier protein (ACP) of the actinorhodin polyketide synthase (PKS) of Streptomyces coelicolor A3(2) was replaced with homologs from the granaticin, oxytetracycline, tetracenomycin, and putative frenolicin polyketide synthase gene clusters. All of the replacements led to expression of functional synthases, and the recombinants synthesized aromatic polyketides similar in chromatographic properties to actinorhodin or to shunt products produced by mutants defective in the actinorhodin pathway. Some regions within the ACP were also shown to be interchangeable and allow production of a functional hybrid ACP. Structural analysis of the most abundant polyketide product of one of the recombinants by electrospray mass spectrometry suggested that it is identical to mutactin, a previously characterized shunt product of an actVII mutant (deficient in cyclase and dehydrase activities). Quantitative differences in the product profiles of strains that express the various hybrid synthases were observed. These can be explained, at least in part, by differences in ribosome-binding sites upstream of each ACP gene, implying either that the ACP concentration in some strains is rate limiting to overall PKS activity or that the level of ACP expression also influences the expression of another enzyme(s) encoded by a downstream gene(s) in the same operon as the actinorhodin ACP gene. These results reaffirm the idea that construction of hybrid polyketide synthases will be a useful approach for dissecting the molecular basis of the specificity of PKS-catalyzed reactions. However, they also point to the need for reducing the chemical complexity of the approach by minimizing the diversity of polyketide products synthesized in strains that produce recombinant polyketide synthases.

PMID: 8468280, UI: 93224438

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[]1: EMBO J 1992 Feb;11(2):785-92 Related Articles, Books, Protein, LinkOut

Cloning of the complete biosynthetic gene cluster for an aminonucleoside antibiotic, puromycin, and its regulated expression in heterologous hosts.

Lacalle RA, Tercero JA, Jimenez A

Centro de Biologia Molecular (CSIC and UAM), Universidad Autonoma, Madrid, Spain.

Puromycin, produced by Streptomyces alboniger, is a member of the large group of aminonucleoside antibiotics. The genes pac and dmpM, encoding a puromycin N-acetyl transferase and an O-demethyl puromycin O-methyltransferase, respectively, are tightly linked in the DNA of S. alboniger. The entire set of genes encoding the puromycin biosynthesis pathway was cloned by screening a gene library from S. alboniger, raised in the low copy number cosmid pKC505, with a DNA fragment containing pac and dmpM. Puromycin was identified by biochemical and physicochemical methods, including 1H NMR, in the producing transformants. This pathway was located in a single DNA fragment of 15 kb which included the resistance, structural and regulatory genes and was expressed when introduced into two heterologous hosts Streptomyces lividans and Streptomyces griseofuscus. In addition to pac and dmpM, two other genes have been identified in the pur cluster: pacHY, which determines an N-acetylpuromycin hydrolase and prg1, whose deduced amino acid sequence is significantly similar to that of degT, a Bacillus stearothermophilus pleiotropic regulatory gene.

PMID: 1537349, UI: 92164669

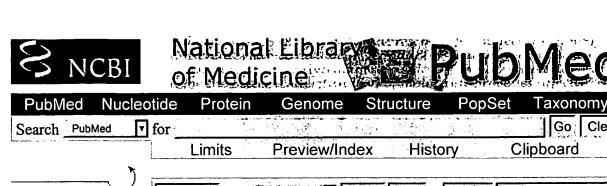
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1: Gene 1994 Dec 30;151(1-2):1-10

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Publyled Services Characterization of the Streptomyces peucetius ATCC 29050 genes encoding doxorubicin polyketide synthase.

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Grimm A, Madduri K, Ali A, Hutchinson CR

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Department of Genetics, University of Wisconsin, Madison 53706.

Text*

The dps genes of Streptomyces peucetius, encoding daunorubicin (DNR)-doxorubicin (DXR) polyketide synthase (PKS), are largely within an 8.7-kb region of DNA that has been characterized by Southern analysis, and gene sequencing, mutagenesis and expression experiments. This region contains nine ORFs, many of whose predicted products are homologous to known PKS enzymes. Surprisingly, the gene encoding the DXR PKS acyl carrier protein is not in this region, but is located about 10 kb distant from the position it usually occupies in other gene clusters encoding type-II PKS. An in-frame deletion in the dpsB gene, encoding a putative subunit of the DXR PKS, resulted in loss of production of DXR and the known intermediates of its biosynthetic pathway, confirming that this gene and, by implication, the adjacent dps genes are required for DXR biosynthesis. This was verified by expression of the dps genes in the heterologous host, Streptomyces lividans, which resulted in the production of aklanonic acid, an early intermediate of DXR biosynthesis.

PMID: 7828855, UI: 95129838

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Tickned Bonder	Characterization of Streptomyces argillaceus genes encoding a polyketide synthase involved in the biosynthesis of the antitumor mithramycin.
	Lombo F, Blanco G, Fernandez E, Mendez C, Salas JA
	Departamento de Biologia Funcional, Instituto Universitario de Biotecnologia de Asturias (I.U.B.A-C.S.I.C), Universidad de Oviedo, Spain.
NEW TUDGENSE T	Mithramycin (Mtm) is an aromatic polyketide which shows antibacterial and antitumor activity. From a chromosomal cosmid library of Streptomyces argillaceus, a Mtm producer, a clone (cosAR7) was isolated by homology to the actI/III region of S. coelicolor and the strDEM genes of S. griseus. From this clone, a 5.3-kb DNA region was sequenced and found to encode six open reading frames (designated as mtmQXPKST1), five of them transcribed in the same direction. The deduced products of five of these genes resembled components of type-II polyketide synthases. The mtm genes would code for an aromatase (mtmQ), a polypeptide of unknown function (mtmX), a beta-ketoacylsynthase (mtmP) and a related 'chain length factor' (mtmK), an acyl carrier protein (mtmS) and a beta-ketoreductase (mtmT1). The involvement of this gene cluster in Mtm biosynthesis was demonstrated by the Mtm non-producing phenotype of mutants generated in two independent insertional inactivation experiments.
	PMID: 8654997, UI: 96257259

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☐ 1: Gene 1993 Aug 16;130(1):107-16

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Hybridization and DNA sequence analyses suggest an early evolutionary divergence of related biosynthetic gene sets encoding polyketide antibiotics and spore pigments in Streptomyces spp.

Blanco G, Brian P, Pereda A, Mendez C, Salas JA, Chater KF

Departamento de Biologia Funcional, Universidad de Oviedo, Spain.

NEW

The whiE gene cluster of Streptomyces coelicolor, which is related to gene sets encoding the biosynthesis of polycyclic aromatic polyketide antibiotics, determines a spore pigment. Southern blotting using probes from three different parts of the whiE cluster revealed related gene sets in about half of a collection of diverse Streptomyces strains. A 5.2-kb segment of one such cluster, sch, previously shown to determine spore pigmentation in Streptomyces halstedii, was sequenced. Seven open reading frames (ORFs), two of them incomplete, were found. Six of the ORFs resemble the known part of the whiE cluster closely. The derived gene products include a ketosynthase (= condensing enzyme) pair, acyl carrier protein and cyclase, as well as two of unidentified function. The seventh ORF diverges from the main cluster and encodes a protein that resembles a dichlorophenol hydroxylase. Comparison with sequences of related gene sets for the biosynthesis of antibiotics suggests that gene clusters destined to specify pigment production diverged from those destined to specify antibiotics early in the evolution of the Streptomyces genus.

PMID: 8344517, UI: 93345807

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